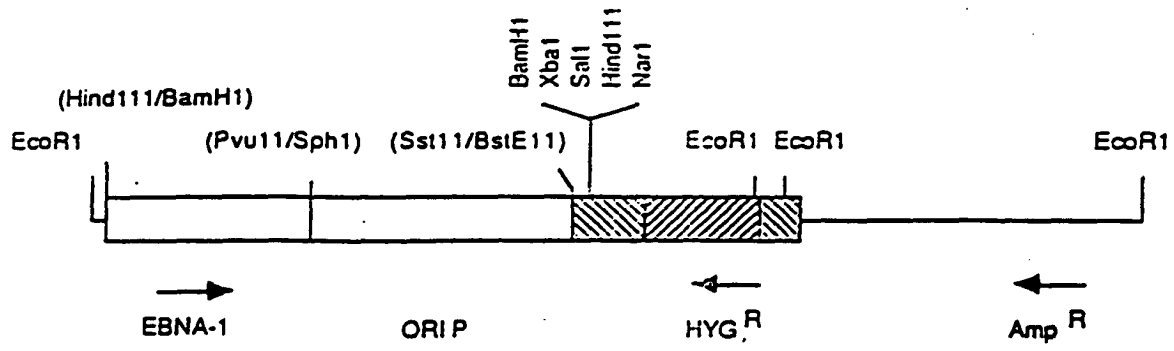
Figure 1 EBV-Based Self-Replicating Expression Vector

p220.2 is a 8952 bp plasmid which encodes for EBNA-1, OriP and Hygromycin resistance. It replicates as plasmid in 143 and HeLa cells. EBNA-1 in this construct is driven off an unknown promoter located in the pBR322 sequences. DNA inserted upstream of EBNA-1 appears to eliminate expression of EBNA-1.



bp		
1-35	——	pBR322
36-2646	▭	EBV EBNA-1 107567-110176 (Baer et. al., Nature 310:1984) Bam H1-Pvu11 fragment. Bam H1 site was blunt-end ligated to the Hind111 site.
2647-4826	▭	EBV OriP 7333-9516 Sph1- Sst11 sites blunt-end ligated to the BstE11 site. (Sugden et.al., MCB 5: 410, 1985)
4827-5460 6488-6747	▨	HSV TK regulatory region (McKnight, S.L., Nucleic Acids Res. 8, 5949, 1980.) Pvu11 fragment ligated into the poisonless pBR322 at Nae 1 site. These sites lost in cloning.
5461-6487	▨	HPH gene (Gritz and Davies, Gene 25:179, 1983) Ban H1 fragment blunt end ligated into the Sma1 and Bgl1 sites in HSV TK sequences.
6748-8952	——	pBR322 poisonless vector (deletion of 1.1 kb in pBR322) confers ampicillin resistance. (Lusky & Botchan, Nature 293:79,1981)

The polylinker from pUC 12 (Sma1-Hae111 fragment) is inserted into a Nar1 site within the HSV TK sequences. The Pst1 site in the polylinker is not unique.

(/) denotes "blunt-end ligations", these sites were not regenerated in cloning.

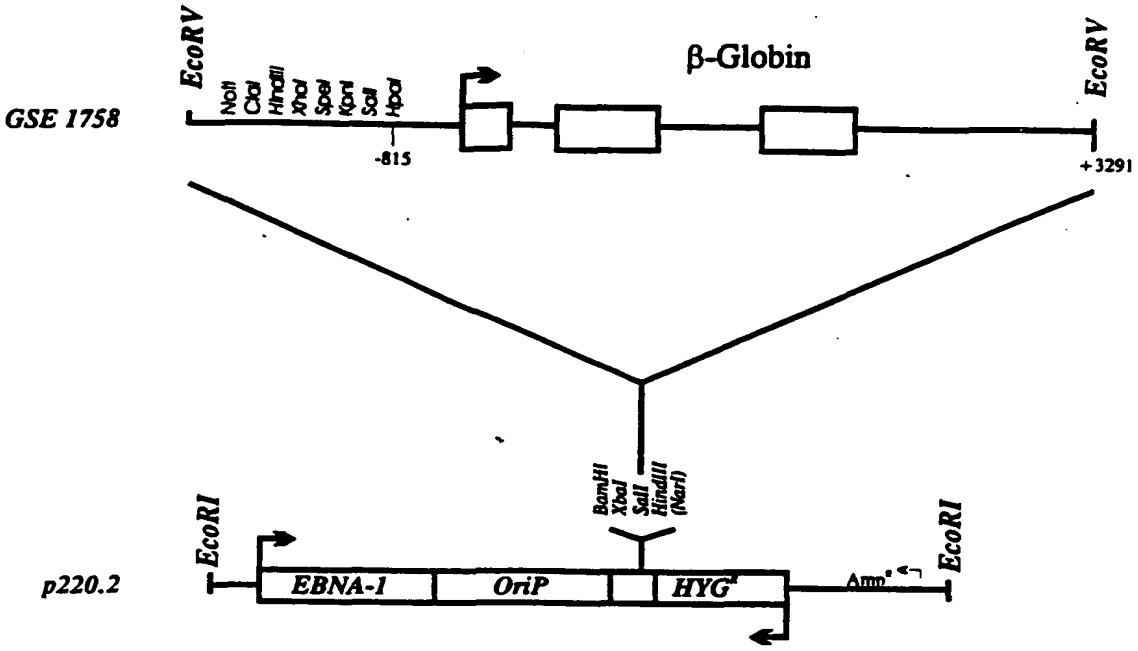


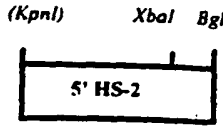
Figure 2: Reporter gene construct.

The β-globin gene extending from a 5' *HpaI* site at -815bp to an *EcoRV* site 1685bp passed the poly(A)-addition site in the plasmid GSE1758 (Talbot et al., 1990) was removed as a 4.1kb *EcoRV* fragment and inserted into a blunted *SalI* site in the polylinker of p220.2 (Figure 1). Note: this cloning step brings a number of extra restriction enzyme sites (including a unique *SalI* site) 5' of the β-globin gene.

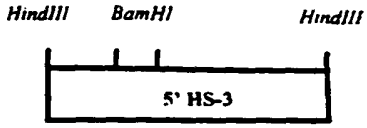
Reference:

Talbot, D., Philipsen, S., Fraser, P. and Grosveld, F. (1990) EMBO J. **9**: 2169-2178.

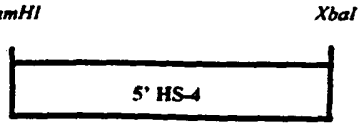
5' HS-2 - 1.5kb KpnI-BglII blunted fragment



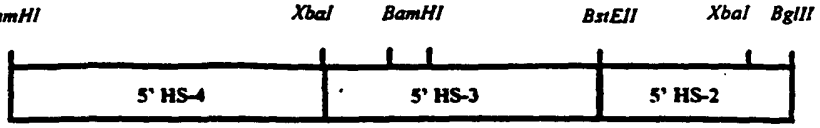
5' HS-3 - 1.9kb HindIII fragment



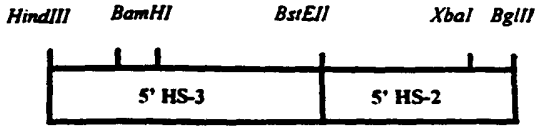
5' HS-4 - 2.1kb BamHI-XbaI fragment



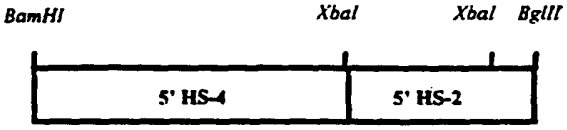
5' HS-4-3-2
5.5kb construct



5' HS-3-2
3.4kb construct



5' HS-4-3
4kb construct



5' HS-4-2
3.6kb construct

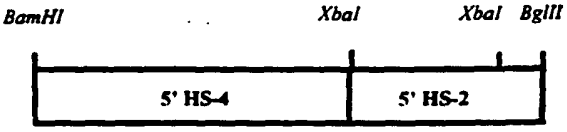


Figure 3: β-Globin LCR hypersensitive site constructs

Multiple hypersensitive site constructs retained the site order found in the wild type β-globin locus. *SalI* linkers were added to both the 5' and 3' ends allowing the DNA to be cloned into the unique *SalI* site in the β-globin-p220.2 reporter vector (Figure 2).

S1 analysis of K5[illegible] cells containing human β -globin on an EBV based vector

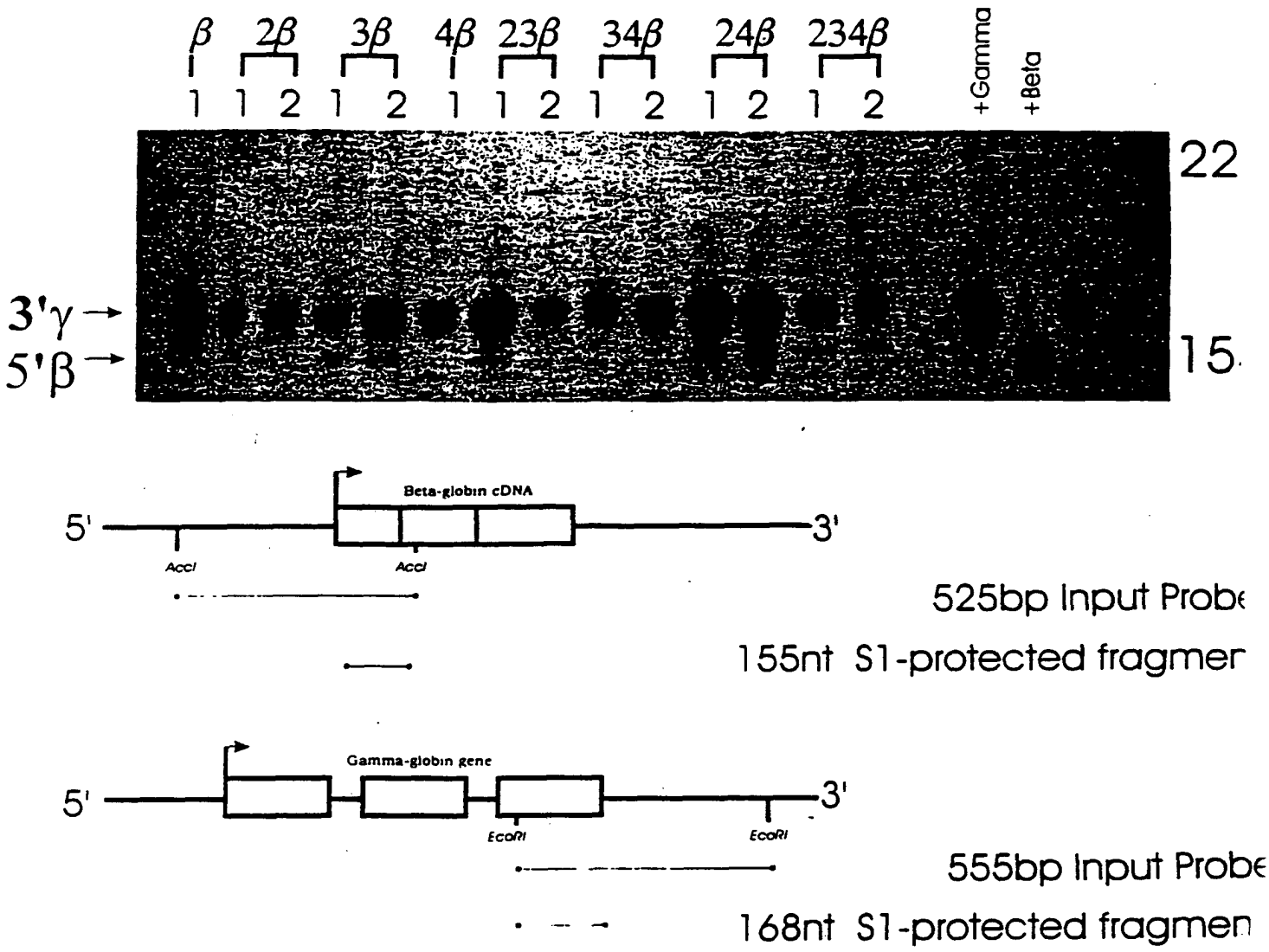


Figure 4: Numbers represent β-globin locus control region DNAseI hypersensitive site combination used.

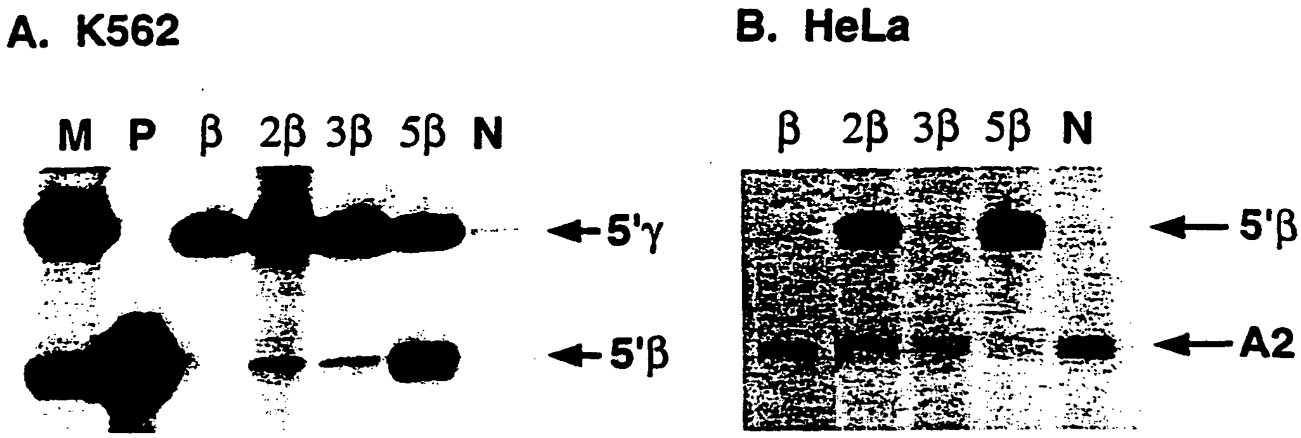


Figure 5.

Fig. 6

Expression Analysis of βLCR/Episome Constructs in K562 Cells